variation from ideal Watson-Crick base pairing. A molecular melt curve also can be used to indicate the degree of binding between one or more test molecules and a target molecule. "Binding" includes not only, e.g., receptor-ligand interactions, but also, e.g., nucleic acid-nucleic acid hybridization interactions and can include both specific and nonspecific interaction. If the test molecules do bind to the target molecule, then their binding can be quantified by the invention. The methods and devices herein are flexible and can be applied to many different types of compounds and molecules. For example, both the target molecule and the test molecules can be any one or more of, e.g., a protein (whether enzymatic or not), an enzyme, a nucleic acid (e.g., DNA and/or RNA, including, single-stranded, double-stranded, or triple-stranded molecules), a ligand, a peptide nucleic acid, a cofactor, a receptor, a substrate, an antibody, an antigen, a polypeptide, monomeric and multimeric proteins (either homomeric or heteromeric), synthetic oligonucleotides, portions of recombinant DNA molecules or chromosomal DNA, portions or pieces of proteins/peptides/receptors/etc. that are capable or having secondary, tertiary, or quaternary structure, etc. The target molecule also optionally interacts with, e.g., co-enzymes, co-factors, lipids, phosphate groups, oligosaccharides, or prosthetic groups.

[0049] Briefly, the methods and devices of the invention enable the construction of and comparison of molecular melt curves. Molecular melt curves are alternatively described as "thermal melting curves", "thermal melt curves", "thermal property curves", "thermal denaturation curves" or "thermal profile curves." Accordingly, an analysis involving the generation of molecular melt curve can also be described as a molecular melt analysis, a thermal melting analysis, a thermal melt analysis, a thermal property analysis, a thermal denaturation analysis, or a thermal profile analysis. In such an analysis, a sample of a target molecule, or target molecules, to be tested is flowed into one or a number of microchannels in a microfluidic device. Optionally, the target molecule is then contacted with one or more test molecules that are screened for possible binding capability with the target molecule and/or with an indicator such as a fluorescence indicator dye or molecule. Optional embodiments of the present invention allow for multiple configurations of, e.g., heat application, flow speed, reagent composition, binding conditions, and timing of all the multiple variants involved.

[0050] Once the test molecule interacts with the target molecule and/or labeling compound, the present invention sets the reaction conditions, in a controllable manner, to a desired temperature (either continuously over a range of temperatures or non-continuously to discrete temperature points). Selected physical properties of the molecules are measured in the microfluidic device and thermal property curves produced from the measurements. The thermal property curves are based upon, e.g., the temperature induced denaturation or unfolding that occurs when the molecules are subjected to heat. Denaturation can include, e.g., loss of secondary, tertiary, or quaternary structure by means of uncoiling, untwisting, or unfolding, disassociation of nucleic acid strands, etc. When target and test molecules bind to one another, e.g., as with receptor-ligand interactions, the conformation of the target molecule is stabilized and the pattern of the temperature induced denaturation is altered or shifted. Comparison of the thermal property curve derived from heating just the target molecule, with the thermal property curve derived from heating the target molecule and test molecule(s) in combination, allows the determination and quantification of any binding between the target molecule and the test molecule(s). The adaptability of the current invention optionally allows both thermal property curves to be run simultaneously in the microfluidic device, as well as optionally running multiple configurations of the binding assay simultaneously (e.g., with different reaction parameters, such as pH, temperature gradient(s), etc.).

[0051] Numerous types of molecules can be assayed by the methods, devices, and systems of the present invention. For example, protein-protein binding reactions can be examined, including, e.g., receptor-ligand, antibody-antigen, and enzyme-substrate interactions. Additionally, interactions between, e.g., amino acid based molecules and nucleic acid based molecules can be examined. Similarly, artificial molecules such as peptide nucleic acids (PNAs) can be monitored, e.g., in interactions of the PNAs with nucleic acids or other molecules. Also, screening for interactions between hydridization probes and nucleic acids, e.g., comprising single nucleotide polymorphisms (SNPs), can be accomplished through use of the current invention. For examples of types of molecular interactions optionally assayed by the invention, see, -e.g., Weber, P. et al., (1994) "Structure-based design of Synthetic Azobenzene Ligands for Streptavidin" *J Am Chem Soc* 16:2717-2724; Brandts, J. et al., (1990) *American Laboratory*, 22:3041+; Gonzalez, M. et al., (1997) "Interaction of Biotin with Streptavidin", *J Biol Chem*, 272(17): 11288-11294; Chavan, A. et al., (1994) "Interaction of nucleotides with acidic fibroblast growth factor (FGF-1) *Biochem*, 33(23):7193-7202; Morton, A. et al., (1995) "Energetic origins of specificity of ligand binding in an interior nonpolar cavity of T4 lysozyme." *Biochem*, 34(27):8564-8575; Kleanthous, C. et al., (1991) "Stabilization of the shikimate pathway enzyme dehydroquinase by covalently bound ligand" *J Biol Chem*, 266(17): 10893-10898; Pilch, D. et al., (1994) "Ligand-induced formation of nucleic acid triple helices." *Proc Natl Acad Sci USA*, 91(20):9332-9336; and Barcelo, F. et al. (1990) "A scanning calorimetric study of natural DNA and antitumoral anthracycline antibiotic-DNA complexes." *Chem Biol Interact*, 74(3): 315-324.

[0052] The actual detection of a change(s) in a physical property of the molecules can be detected in numerous methods depending on the specific molecules and reactions involved. For example, the denaturation of the molecules can be tracked by following fluorescence or emitted light from molecules in the assay. The degree of, or change in, fluorescence is correlational or proportional to the degree of change in conformation of the molecules being assayed. The methods and devices of the invention allow for various methods of exciting the molecules involved in the assay, through use of, e.g., lasers, lights, etc. The fluorescence can be intrinsic to the molecules being assayed, e.g., from tryptophan residues in the molecules, or extrinsic to the molecules being assayed, e.g., from fluorophores added to the assay mixture in the microfluidic device. The change(s) in fluorescence or emitted light can optionally be detected in a number of ways according to the specific needs of the assay desired. For example, a charge-coupled device is utilized as an optional part of the device.

[0053] The change in fluorescence of emitted light indicates a change in conformation of the target molecule and from which the thermal property curve is constructed. Displacement or shift of the thermal property curve when the target molecule is in the presence of a test molecule allows